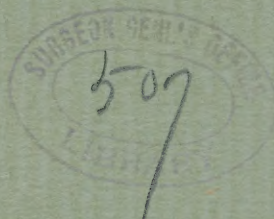# ON THE APPEARANCE OF CARBAMIC ACID IN THE URINE AFTER THE CONTINUED ADMINISTRATION OF LIME WATER, AND THE FATE OF CARBAMIC ACID IN THE BODY.

BY JOHN J. ABEL, M. D., *Professor of Pharmacology, the Johns Hopkins University.*

[*Read before the Johns Hopkins Hospital Medical Society.*]

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It has long been known that an excess of lime water added to the food will render the urine alkaline, but so far as I can ascertain no inquiry has ever been made into the nature of this alkalinity nor into the chemical conditions accompanying it. My attention was first directed to this question by an incident that occurred in the practice of Prof. V. C. Vaughan of the University of Michigan. A mother for whose infant lime water had been prescribed and who had administered it very freely for some months, consulted Prof. Vaughan because of the ammoniacal odor of the child's linen when once it had been moistened with urine. On examination the urine was found to be strongly alkaline and to yield free ammonia. No inflammation of the bladder or other abnormal condition could be detected as a cause for the ammoniacal urine. That a perfectly fresh urine voided from a healthy bladder should give off free ammonia is certainly a fact that calls for examination. Assisted by Dr. Archibald Muirhead, I accordingly performed a series of experiments the results of which I am inclined to believe will be of interest to you. We began our experimental inquiry with a study of the urine of healthy dogs about a year old fed on bone-free meat and fat and found, as was to be expected, that with such a diet the urine is generally acid in reaction, though sometimes amphoteric or faintly alkaline immediately after meals. Such neutral or alkaline urines, strange to say, often yield very small quantities of free ammonia, especially, so it appeared to us, when the dogs were fed on the ordinary refuse of the butchers' shops—that is, with meat containing much bone.

Having convinced ourselves that only very small quantities of free ammonia when any are given off by the normal urine of the dog, we proceeded to examine the effect of feeding large doses of calcium hydrate. Thick cream of lime was mixed in equal parts with bran or crumbed bread, the mixture enclosed in large gelatine capsules and then placed far back on the tongue of the dog who was thus forced to swallow the bolus. By feeding twice a day it is an easy matter to administer daily to a large dog 8 to 10 grams of slaked lime, and this without causing any symptoms of disturbed digestion or any other untoward effects beyond more or less constipation, if only care be taken to administer the lime immediately after meals. When the dogs have been taking such large quantities of lime for five or six days, the freshly-voided urine exhibits the characteristics of the specimens that I have brought here to show you.

You will observe that the urine is strongly alkaline, a strip of red litmus paper dipped into it at once turns a deep blue and a moistened strip of red litmus suspended from a cork in the mouth of the flask also turns blue in the course of a few minutes. You will also observe that the urine is very turbid, that a film of crystals forms on the surface and that a heavy deposit of crystals lies on the bottom of the flasks. This increased turbidity, the surface film and the heavy sediment are almost entirely due to the deposition of crystals of ammonio-magnesium phosphate in all stages of growth. I am well aware that triple phosphates are often to be found in the urine of cats and dogs fed on meat, but they are never deposited so constantly and in such quantities as in the urines under discussion. It must be noted, too, that the triple phosphates are to a large extent precipitated in the bladder itself in the case of dogs fed on lime, for the urine is very turbid at the time that it is voided. A microscopic examination of the surface film often demonstrates the presence of biscuit-shaped crystals, soluble in acetic or hydrochloric acid, with the evolution of much gas. These biscuit-shaped crystals, therefore, are composed of calcium carbonate and they are only to be found when very much lime has been taken and relatively little water has been drunk.

In order to estimate the quantity of ammonia emitted spon-

taneously by the urine of healthy dogs both before and after feeding lime we proceeded as follows. Twenty-five cubic centimeters of the freshly voided urine were placed in a bowl under a dish containing ten cubic centimeters of normal sulphuric acid and both covered with a small bell jar as in the Schlösing method for determining ammonia. A strip of moistened red litmus paper was hung from the upper part of the bell jar to indicate the time when the absorption of the NH_3 should be complete. Since this absorption requires from five to seven days, a drug that will prevent fermentation must be added to the urine. We employed thymol for this purpose, first breaking it up into small pieces, adding liberally of these and stirring them into the urine before covering it with the bell jar. Check experiments with normal urines were always made in order to exclude the possibility of ammoniacal fermentation. In all cases the urines remained perfectly clear with the exception of the surface layer of thymol and the heavy deposit of triple phosphates. When the strip of red litmus in the upper part of the bell jar that serves as indicator again changes from deep blue to pink, the absorption of ammonia is so far complete that the residual titration of the normal sulphuric acid used for absorbing the ammonia may be undertaken. In a series of such absorption experiments it was found that the following amounts of ammonia had been given off by specimens of lime urine.

Dog No. 1,	20 cc.	of morning urine yielded			0.0033	NH_3	in	5	days.
"	"	40	"	"	"	"	0.0074	"	" 9	"
Dog No. 2,	25	"	"	"	"	0.0054	"	" 7	"
"	"	25	"	"	"	"	0.0031	"	" 7	"
"	"	25	"	"	"	"	0.0044	"	" 7	"
"	"	25	"	"	"	"	0.0070	"	" 7	"
"	"	25	" of evening	"	"	0.0072	"	" 7	"

Without lime the urine yields, as I said before, only little or no ammonia. Thus the highest amount ever obtained from the normal urine (25 cc.) of dog No. 1, under the conditions of absorption just stated, was 0.0016 gram. The normal urine of dog No. 2 gave off no ammonia to the standard acid solution during the time that he was under observation. The urine of a third dog which was also more often devoid of free ammonia than otherwise however once yielded 0.00195 gram of ammonia.

These absorption experiments therefore demonstrate that there is regularly a large spontaneous emission of NH₃ from the lime urines, that is, large in comparison with the total amount of ammonia contained in normal urine. A glance at the tabulated results that will be given later, in which the total ammonia in 20 cc. of urine as well as in the 24 hours urine is set down, will make this point very evident.

As the ammonia of urine is ordinarily present in the form of salts, it was of interest to determine what proportion of the ammonia of lime urines was present as free ammonia, and what proportion in the usual form of ammonia salts. In order to solve this question we placed 40 cc. of urine that had stood nine days under the bell jar and had yielded 0.0074 gram NH₃ in that time, into an ordinary exsiccator for another ten days, occasionally adding water to make good the absorption. At the expiration of this time the urine was filtered free of the thymol and sediment of phosphates and treated for ammonia by the Schmiedeberg process. Not a trace of ammonia could be discovered,—hence this particular specimen of urine contained no ammonia in the ordinary form of stable salts of ammonia, all of the ammonia being present as free ammonia. Later experiments have, however, convinced me that this is not always the case, and an investigation is now being carried on in my laboratory on the excretion of ammonia under various clinical conditions in which this question will again be discussed. Leaving aside, then, the question as to the relative amount of ammonia present in the form of free ammonia and stable salts as for the present still *sub judice*, we come to a second question of pharmacological interest, and I should like to digress far enough to treat it here. Does more or less ammonia leave the body in 24 hours after feeding with lime than before, other conditions such as food and drink remaining constant? The experiments of Coranda,* Munk and Salkowski† have taught us that salts of the fixed alkalis, sodium carbonate for example, lower the amount of ammonia excreted in the twenty-four hours urine. Now sodium carbonate, while it causes the urine to become alkaline in reaction, does not, as I have demonstrated, cause the appear-

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*Archiv f. experimentelle Pathol. u. Pharmakol., Bd. 12, pp. 76–96.
† Archiv f. pathol. Anat. 1877, Bd. LXXI. Maly's Jahresber. der Thier. Chemie, Bd. VII (1877), pp. 192–4.

ance of free ammonia. This marked difference in the influence of the two drugs on the character of the alkalinity of the urine caused us to test the influence of lime on the total ammonia excretion. Ammonia determinations were made twice a day by the Schmiedeberg* method for a number of consecutive days before and after feeding with lime. Briefly stated, this method, which is to be preferred to the Schlösing method for dog's urine, involves the following manipulations. Twenty cc. of the urine are precipitated with platinum chloride and 5 to 6 volumes of a mixture composed of two volumes of absolute alcohol and one volume of ether, and allowed to stand in a cool place for 24 hours. The precipitate is then collected and thoroughly washed with ether and alcohol, and after drying is reduced with zinc and hydrochloric acid at a moderate temperature. When the precipitate of platinum salts has been entirely decomposed and the filtrate from it is colorless the latter is distilled with magnesium oxide, the distillate is caught in a properly arranged flask containing 10 cc. of normal sulphuric acid and titrated back with $\frac{1}{4}$ normal sodium hydrate solution.

It did not seem necessary to put the dog into nitrogenous equilibrium, for food and other conditions remaining constant, any strikingly large variation in ammonia excretion during the lime treatment could only be referred to the lime as its cause. The food consumed by the dog consisted of Spratt's biscuits and was carefully weighed before and during the period of lime treatment; the dog was also weighed before beginning the experiments at the end of the no-lime series of analyses and again at the close of the lime series. During the time of the analyses the dog was confined in a roomy cage, the urine, however, was caught three times a day, the dog having been trained to urinate into a glass vessel when presented. In these quantitative experiments in which Spratt's biscuits were fed no constipation followed the lime treatment for the reason that this food tends to cause looseness of the bowels. The tables, pages 6 and 7, contain the results of the ammonia estimations during the periods of the no-lime and of the lime treatment.

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* Archiv f. exp. Pathol. u. Pharmakol., Bd. 7, p. 166, in paper by F. Walter : Untersuchungen über die Wirkung der Säuren auf den thierischen Organismus.

EXCRETION OF NH₃ DURING PERIOD OF NO-LIME.

	Amount of urine in cc.	Reaction.	NH_3 in 20 cc. of urine.	NH_3 in noon and evening urines.	NH_3 in morning urine.	NH_3 in the 24 hours urine.	One kilogram of Spratt's biscuits consumed daily.
August 14.	noon } evening } 359. morning 180.	weakly alkaline. } acid. acid.	0.01008 0.01898	0.13093	0.17082	0.3517	
August 15.	noon } evening } 350. morning 250.	weakly alkaline. " acid.	0.0094 { Not determined. Accident.	0.1645	0.1487*	0.3132	
August 16.	noon } evening } 400. morning 280.	weakly alkaline. " acid.	0.0044 0.01058	0.0880	0.1481	0.2361	
August 17.	noon } evening } 337. morning 250.	weakly alkaline. " acid.	0.00638 0.0116	0.1075	0.1450	0.2525	
August 18.	noon } evening } 281. morning 218.	weakly alkaline. " acid.	Not determined. Accident. 0.01293	0.13197†	0.14093	0.2729	
August 19.	noon } evening } 253. morning 242.	weakly alkaline. " acid.	0.00924 0.01024	0.11688	0.1239	0.2407	

* This number was found by using an average for the NH_3 in 20 cc. of morning urines.

† This number was found by using an average for the NH_3 in 20 cc. of the evening and noon urines. The occasional use of an average or mean value in calculating the NH_3 in half of the twenty-four hours urine cannot appreciably affect the results of such a long series of analyses as the above, where two separate ammonia determinations are made in the twenty-four hours.

EXCRETION OF NH₃ DURING THE PERIOD OF FEEDING LIME.

	Amount of urine in cc.	Reaction.	NH₃ in 20 cc. of urine.	NH₃ in noon and evening urines.	NH₃ in morning urine.	NH₃ in the 24 hours' urine.	Weight of Spratt's biscuits consumed, in kilogram.
August 23.	noon } 365. evening } morning 305.	weakly acid. } alkaline. } acid.	0.00621 0.00924	0.11643	0.14091	0.2573	0.635
August 24.	noon } 210. evening } morning 257.	alkaline. } " } weakly acid.	0.00386 0.01092	0.04053	0.14032	0.1808	0.710
August 25.	No analyses made because the morning urine not yet strongly alkaline.						
August 26.	noon } 360. evening } morning 125.	alkaline. } " }	not determined. 0.01612	0.1335*	0.1007	0.2342	0.830
August 27.	Results damaged owing to the occurrence of an accident while catching the urine.						0.770
August 28.	"	"	"	"	"		0.625
August 29.	noon } 240. evening } morning 200.	strongly alkaline gives off much NH₃. " "	0.00672 0.01008	0.08232	0.1008	0.1831	0.875
August 30.	noon } 255. evening } morning 200.	strongly alkaline gives off much NH₃. " "	0.0084 0.00688	0.1051	0.0688	0.1739	0.625
August 31.	noon } 362. evening } morning 145.	strongly alkaline gives off much NH₃. " "	not determined. 0.0049	0.0887	0.0538*	0.1425	0.615
September 1.	noon } 200. evening } morning 185.	strongly alkaline gives off much NH₃. " "	0.01192 0.00806	0.1192	0.0745	0.1937	0.830
September 2.	noon } 270. evening } morning 205.	strongly alkaline gives off much NH₃. " "	not determined. 0.0047	0.10017	0.04817*	0.1483	0.900

* These values were found by using averages for the morning or afternoon urines, as the case might be, as already explained.

The dog's weight on the 8th of August was found to be 45.5 kg. On the 22nd of August the dog's weight was again taken and was found to be 47.25 kg. On the 6th of September some days after the close of the lime period it was found to be 47.5 kg. On the 21st of August milk of lime was mixed for the first time with the food. A glance at the columns in which the total NH_3 excretion for the 24 hours is put down will make it evident how marked is the falling off in the ammonia output when once the dog's system is thoroughly under the influence of slaked lime. If we entirely neglect the circumstance that the dog increased more in weight during the first or no-lime period than during the time of the lime treatment, since it is impossible to determine by calculation what relation this fact bears to the ammonia excretion, and if we assume that the NH_3 excretion is proportional to the amount of food digested, and if we furthermore make the proper correction in the numbers that represent the NH_3 output during the lime period, we shall still have a large unaccounted-for deficit in NH_3 excretion. Thus during six consecutive days of no-lime and a total consumption of six kg. of Spratt's biscuits the total NH_3 output $=$ 1.6671 grams NH_3; during *five consecutive days of lime treatment* (from the 29th of August to the 2nd of September inclusive), and during a total consumption of 3.845 kg. of Spratt's biscuits, the total NH_3 output $=$ only 0.8415 gram. For six days at the same rate of food consumption and ammonia excretion the NH_3 output would have amounted to 1.0124 grams. If the dog had consumed in these six days of lime treatment 6 kg. of biscuits instead of 4.614 kg., and if the NH_3 excretion had increased in proportion, we should have had an output of only 1.3131 grams. If this amount is compared with the output actually obtained in the six days of the no-lime period, viz. 1.6671 grams, we find that 21.23 per cent. less NH_3 is excreted in the 24 hours when dogs are fed with large quantities of slaked lime then when no lime is given. That the smaller amount of food taken during the period of treatment was not due to the lime taken was demonstrated by the fact that the dog did not again consume 1 kg. of biscuits after the cessation of the treatment. As has been remarked before, nothing abnormal could be detected in the dog's condition during the time of

treatment. It must be evident, therefore, that lime-water if given in large quantities and continuously will lower the output of ammonia as markedly as do the carbonates of the fixed alkalis. I may remark in passing that such experiments as the above are the counterpart of those performed by Walter,[*] Hallervorden,[†] Coranda[‡] and Salkowski,[§] who found that the administration of hydrochloric acid greatly increased the ammonia output of the 24 hours' urine.

There are a few more questions touching this ammoniacal state of the urine that must be referred to. Thus, the morning urine for some time after the appearance of the free ammonia owes its alkalinity entirely and alone to the ammonia. A strip of red litmus moistened with morning urine, if allowed to dry in an atmosphere free from the fumes of ammonia and volatile acids, or placed under the bell jar of an exsiccator, quickly takes on its original red color, thus demonstrating that the alkalinity of the urine in question is due solely to the ammonia emitted by it. Such a specimen of urine, if evaporated somewhat on the water-bath and then restored to its original volume by the addition of water, will show a weakly acid reaction in place of its former markedly alkaline reaction. The noon and evening urines owe their alkalinity partly to stable salts, for a strip of red litmus paper dipped into them retains its blue color on drying. The behavior of the morning urine,[||] then, does not support the opinion that the ammonia emitted by it is driven out by some salt of the fixed alkalis or alkaline earths, otherwise one would suppose that a strip of red litmus would remain blue after the evaporation of the ammonia. We must look, therefore, to some unstable compound that breaks down of its own accord as the source of the free ammonia. One such compound known to us is sodium ammonium phosphate, $PO\begin{cases} ONa \\ ONH_4, \\ OH \end{cases}$ found in guano and in inspissated urine, an aqueous solution of which liberates ammonia even at ordi-

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[*] Archiv f. exp. Pathol. u. Pharmakol., Bd. VII, pp. 148–178.

[†] *Ibid.* Bd. XII, pp. 237–75.

[‡] *Ibid.* Bd. XII, pp. 76–96.

[§] Archiv f. pathol. Anat. u. Physiol., Bd. 58, p. 23.

[||] After long feeding with lime the morning urine behaves more like the day urines.

nary temperature in accordance with the following formula

of decomposition: $PO \begin{cases} ONa \\ ONH_4 \\ OH \end{cases} = PO \begin{cases} ONa \\ OH \\ OH \end{cases} + NH_3$. But we

shall give reasons that make it more than probable that the ammonia about whose source we are concerned does not leave the kidney in the form of this phosphate.

We have thus far dealt with the following characteristics of lime urine: the deposition of triple phosphates while the urine is still in the bladder, the spontaneous liberation of much ammonia, its high alkalinity, and the presence of spherules of calcium carbonate. The addition of a few drops of strong hydrochloric acid to a specimen of lime urine generally causes a marked effervescence. That the freshly voided urine contains much carbon dioxide is shown by passing a current of washed air through it and then into a clear solution of barium hydrate, and this may be kept up for many hours without exhausting the supply of CO_2 in the urine. Unfortunately no quantitative analyses were made of the amount of CO_2 that can be pumped out of such a lime urine. Boiling the urine throws down a granular sediment that is dissolved with effervescence on the addition of an acid, and therefore contains $CaCO_3$. Has the frequent appearance of spherules of $CaCO_3$ in the urinary sediment and its high content of $CaCO_3$ in a state of solution any connection with the large amount of ammonia given off by the lime urine? or is the $CaCO_3$ that is thrown down when the urine is boiled present only in the form of the soluble acid salt, $Ca(HCO_3)_2$? If the latter assumption holds good, then no further precipitation of $CaCO_3$ should occur on boiling a urine from which all the bicarbonate of calcium has previously been removed. To accomplish this, one has but to add some freshly prepared milk of lime and shake vigorously for ten or fifteen minutes, then allow to settle for half an hour, then filter and add to the filtrate a few drops of concentrated solution of calcium chloride to decompose any ammonium carbonate that might perchance have escaped the decomposing action of the milk of lime, then add a knife-point full of freshly prepared crystalline calcium carbonate which has been kept under distilled water in a perfectly tight bottle, again shake vigorously for ten minutes, allow to settle for half an hour and again filter. The

knife-point full of crystalline calcium carbonate is added for the purpose of inducing any amorphous or semi-crystalline calcium carbonate that may have remained in solution after the first shaking with milk of lime to fall out as crystalline calcium carbonate. This method, if carefully followed out with properly prepared reagents, will remove all the bicarbonate of calcium from an ammoniacal solution containing it, such as the lime urines in question. But if the urine from one of the lime-fed dogs is treated in this way it will still deposit calcium carbonate on boiling, and the precipitate formed at the bottom and sides of the test-tube will dissolve with effervescence on the addition of an acid. A few bubbles of CO_2 may be obtained from a normal urine treated in this way if the shaking-out methods that we have outlined are carelessly employed, but the much more marked evolution of CO_2 obtained when a lime urine is similarly treated cannot be referred to bicarbonate of calcium that has escaped precipitation. One must conclude, therefore, that the lime urine contains in solution a compound that is not precipitated by the reagents that throw down bicarbonate of calcium, but that it agrees with the bicarbonate in its inability to withstand boiling, like it depositing $CaCO_3$ when its aqueous solutions are heated. This compound has been shown by Mr. Muirhead and myself to be calcium carbamate, a salt remarkable for the instability of its aqueous solutions even at ordinary temperatures. Thus, a clear filtered solution of this salt remains clear at ordinary temperatures for a few minutes only, soon becomes turbid from the deposition of calcium carbonate, and gives off ammonia and CO_2. At room temperature this decomposition is not complete, that is, not all the carbamate in solution breaks up at once, for when once NH_3 has appeared it exercises an inhibitory influence on the further decomposition so that some of the salt may remain undecomposed for a long time. On heating to about $55°$, or on boiling, the dissociation is rapid and complete. The following equation illustrates the manner of this decomposition :

$$\left(CO{<}_O^{NH_2} \right)_2 Ca + H_2O = CaCO_3 + 2NH_3 + CO_2.$$ Bearing the

properties of calcium carbamate in mind, we can explain all the peculiarities of the lime urine, namely, the presence of free

ammonia, of much CO_2, the frequent occurrence of $CaCO_3$ in the urinary deposit, the constant and large precipitation of ammonio-magnesium phosphate in the bladder, and the presence of calcium carbonate in solution after removing any bicarbonate of calcium that may have been present. A connection is therefore most clearly established between these various occurrences if we can prove that calcium carbamate is really present in the lime urines. The behavior of the lime urines toward boiling after the previous removal of any bicarbonate of calcium possibly present, made us suspect the presence of the carbamate, and encouraged us to attempt its isolation. To this end we treated from four to five litres of the urine of dogs that were being fed on meat and lime, by a method first suggested by Drechsel* for the isolation of carbamic acid from the urine. Briefly stated, this method consists in expelling the ammonia of the urine with milk of lime, and getting rid of any ammonium carbonate or bicarbonate of calcium that may be present in the way already described, then precipitating in the cold with at least three volumes of cold absolute alcohol, allowing to stand on ice for fifteen hours, collecting the precipitate as rapidly as possible by filtering under pressure, redissolving it when dry in ammonia, and reprecipitating with cold absolute alcohol by the fractional method. The third fractional precipitate with absolute alcohol was allowed to stand on ice for fifteen hours, the precipitate collected by filtering under pressure, washed with absolute alcohol and ether to remove all traces of moisture and ammonia and then dried *in vacuo* over sulphuric acid. This final precipitate occurs, when dry, in the form of white porous pieces that yield when pulverized a slightly yellowish powder almost entirely soluble in water. Its aqueous solution behaves entirely like solutions of calcium carbamate synthetically† prepared in the laboratory. Thus, the clear filtrate becomes turbid in a few moments, throwing down crystalline calcium carbonate and giving off ammonia. On heating to about 55° C., or on boiling, this decomposition takes place immediately. Unfortunately this white powder that we have isolated from the urines is not pure calcium carbamate, it consists in large
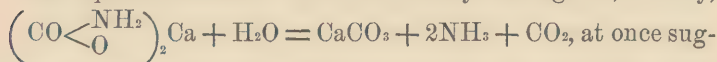
* Drechsel u. Abel, Archiv f. (Anat. u.) Physiol. 1891, p. 238.
† Journ. f. prakt. Chem. (2), Bd. 16, pp. 188–92.

part of conjugate sulphates of calcium, and according to Nencki and Hahn* who have more recently examined it, also contains small quantities of a salt of acetic acid.

The equation for the decomposition of calcium carbamate in an aqueous solution that has already been given, namely, $\left(CO{<}_O^{NH_2} \right)_2 Ca + H_2O = CaCO_3 + 2NH_3 + CO_2$, at once suggests a method of gravimetric analysis for this salt, even though other compounds be present, provided none of these yield ammonia or calcium carbonate when the solution is boiled. That is, if a solution of this salt be decomposed by boiling, and the ammonia arising from its decomposition be distilled off and caught, we ought to find two molecules of ammonia to one of calcium carbonate left as a crystalline sediment in the flask. To test the question we dissolved 0.6803 gram of an impure specimen of calcium carbamate that had been prepared synthetically some months before and which had therefore largely broken down, in water, filtered off quickly from the large insoluble residue of calcium carbonate directly into the decomposition flask and applied heat. The ammonia given off was taken up in hydrochloric acid and estimated as a platinum double salt, the sediment of calcium carbonate in the decomposition flask was collected, dried, weighed, incinerated and estimated as calcium oxide. We found 0.0265 gram CO_2 : 0.02029 gram of NH_3. The proportion $CO_2 : 2NH_3$ demands for 0.0265 CO_2, an amount of NH_3 represented by 0.02047 gram, showing a very close agreement. These results prove that this analytic method will enable us to demonstrate the presence of this salt in solution, provided, as we have said, no other compounds that yield either ammonia or calcium carbonate on boiling are present. We have made a number of analyses of the crude carbamate isolated by us from lime urines, but none of them have been satisfactory from the quantitative point of view. In all cases the amount of $CaCO_3$ found was far in excess of that required by the proportion $CO_2 : 2NH_3$. Thus, in one experiment we found 0.0238 $CO_2 : 0.01018$ NH_3, but 0.0238 CO_2 requires 0.01839 NH_3. In a second analysis we found 0.0211 CO_2 and 0.0124 NH_3. Our

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* Archiv f. experimentelle Pathologie und Pharmakologie, Bd. 32 pp. 198-9.

proportion, however, demands for 0.01211 CO_2 an amount of NH_3 represented by 0.0163. It is perhaps permissible to assume that a basic salt had been formed by the action of the milk of lime on the normal carbonate during the tedious process of isolation already described. The basic salt would have the formula $H_2N.CO.O.Ca.OH$, its aqueous solutions would decompose on boiling according to the following equation, $H_2N.CO.O.Ca.OH = CaCO_3 + NH_3$, and its presence in varying proportion would therefore cause a variation in the amount of CO_2 found on decomposing the crude product isolated from the urine. A fuller discussion of this question and more complete details of the above analyses will be found in our previous papers on this subject.* Whatever opinions may be held as to the presence of a basic carbamate, it is certain that the powder isolated by us contains as a further impurity an unknown compound which also deposits $CaCO_3$ on boiling but which yields no ammonia. We know this from the behavior of the filtrate of a solution of the crude carbamate from which all traces of carbamic acid have been removed by boiling till no more ammonia is given off. If such a filtrate is again subjected to a half-hour's boiling, or is allowed to stand for some hours, calcium carbonate is again deposited, though only in small quantities, while no more ammonia is liberated. We have, therefore, sufficient explanation for the high percentage of calcium carbonate found by us and for the unsatisfactory outcome of our quantitative analyses. But although the quantitative results are far from satisfactory, the behavior of aqueous solutions of the crude product so frequently referred to is so entirely like that of a solution of synthetically prepared carbamate, especially in respect to the rapid deposition of $CaCO_3$ and the liberation of NH_3, that we are justified in concluding that the lime urines contain calcium carbamate, and we are all the more justified in this conclusion since a qualitative analysis of the powder demonstrates nothing that could otherwise explain the contemporaneous appearance of $CaCO_3$ and NH_3 on boiling.

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*Abel u. Muirhead : Archiv f. exp. Pathol. u. Pharmakol., Bd. 31, pp 21-3 ; Drechsel u. Abel : Archiv f. (Anat. u.) Physiologie, 1891, pp. 240-2. See also Hahn, Massen, v. Nencki and Pawlow in Archiv f. exp. Pathol. u. Pharmakol., Bd. 32, pp. 197-200, or Archives des Sciences Biologiques, St. Pétersbourg, t. I, no. 4, pp. 401-95.

It therefore only remains for us to discuss the question whether the carbamate found by us may not be an artificial product, the result of the various manœuvres employed in its isolation. Every normal urine contains small quantities of CO_2[*] either in the free state or in combination, as well as salts of ammonia from which the ammonia is set free when the urine is shaken with milk of lime, and hence it might be inferred that we really have all the conditions for the artificial formation of carbamic acid. Drechsel[†] has demonstrated in his research on the oxidation of glycocoll, leucin and tyrosin that carbamic acid is formed wherever CO_2 and NH_3 meet in the nascent condition. But the circumstances in the two experiments differ widely, for the addition of an excess of milk of lime to the urine binds the small amount of CO_2 that is present at the same time that it liberates the NH_3; certainly these chemical conditions cannot be likened to those met with in the oxidation experiments just referred to, in which compounds containing nitrogen and carbon are oxidized in alkaline solutions, and in which nascent NH_3 and CO_2 are able to act on each other. They differ, too, from those obtaining in the ordinary method of preparing calcium carbamate, namely, the passing of a continuous current of CO_2 into a concentrated solution of ammonia holding milk of lime in suspension. The opinion that the calcium carbamate found by us is not an artificial compound produced in the course of our manipulations, but that it is excreted as such by the kidneys, also receives support from the following experiment. A liter and a half of human urine of acid reaction was treated in the manner already described for the isolation of carbamic acid. The final product was dissolved in water, filtered into a test-tube and boiled; a little calcium carbonate was deposited, but no ammonia was given off. The urine in question, therefore, contained no carbamic acid and the manipulations employed in its isolation are not capable of causing its appearance. It is not to be inferred, however, that an acid urine cannot contain carbamic acid. Nencki and Hahn[‡] have met with it in an acid urine of the horse.

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[*] Pflüger's Archiv, Bd. II., p. 156, and Bd. VI., p. 93.

[†] Journal f. prakt. Chemie (2), Bd. XII., p. 422.

[‡] See Hahn, Massen, v. Nencki and Pawlow, Archiv f. exp. Pathol. u. Pharmakol., Bd. XXXII., p. 200.

It will be remembered that we occasionally found small quantities of free ammonia in the urine of dogs fed on the ordinary refuse of the butchers' shops even when no lime had been mixed with their food. It seemed worth while also to isolate the carbamic acid from such urines. The yield was far less than when lime was mixed with the food, the final product* isolated from the former urines furnishing only $\frac{1}{3}$ as much calcium carbamate as the latter.

Do the experiments that we have detailed apply to human urine? We are prepared to think that they do, and we are confident that all cases of persistent and marked alkalinity of the urine due to long administration of much lime water will be found on examination to be instances of ammoniacal urine due to the presence of a salt of carbamic acid. We were allowed through the kindness of a friend to try the following experiment. A four-year-old boy was given two teaspoonsful a day of very thick cream of lime well distributed in his milk and other food. No digestive disturbances were observed to follow the administration of this quantity of lime but on the evening of the third day the child's urine exhibited all the characteristic reactions of a dilute solution of calcium carbamate. It goes without saying that the administration of lime water even in large quantities would have required a much longer time to produce a like effect.

This case together with the one cited at the opening of this paper suffice to show that so simple a drug as lime-water may cause hitherto unsuspected changes in the urine. Whether the ammonia that is given off by these urines may give rise to any clinical symptoms, such as an increased irritability of the bladder, we are not yet able to say.

In concluding this part of the subject I may be permitted for the sake of clearness to give a brief resumé of the results.

1. The urine of dogs fed on meat becomes strongly alkaline when slaked lime is mixed with the food, and gives off ammonia and carbon dioxide spontaneously.

2. It contains absolutely less ammonia in the 24 hours than normal urine.

3. It always contains a calcium salt in solution which is not

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*Abel and Muirhead, *loc. cit.*, p. 23.

bicarbonate of calcium, and which decomposes with precipitation of calcium carbonate if the urine be allowed to stand.

4. The lime urine exhibits all the characteristics of a weak aqueous solution of calcium carbamate, and a white powder may be isolated from it which behaves in every way like synthetically prepared calcium carbamate, except that it gives less accurate results on being subjected to quantitative analysis.

5. Human urine behaves exactly like that of the dog when large quantities of lime have been taken and it likewise contains calcium carbamate.

Why carbamic acid should appear in such noticeable quantities after the administration of lime we can only surmise. It seems plausible to argue that the body avails itself of its readily soluble calcium salt to get rid of the excess of calcium that has been absorbed. That calcium is absorbed in not inconsiderable quantities has been repeatedly demonstrated, and that there are in the urine but few acids that can form soluble calcium compounds is apparent, but in the present state of our knowledge we can furnish no adequate explanation for the appearance of this acid under the circumstances described in this paper.

The fact that carbamic acid is an important intermediate product of the metabolism of the body gives to its appearance in the urine a more than merely clinical or pharmacological importance. Recent researches have made it more than probable that it is the chief immediate precursor of urea, and that it therefore plays a great part in the complicated chemical processes to which the proteids of our food are subjected before their nitrogen is eliminated in the urine.

As no other theory of the formation of urea in the body has as good an experimental foundation as this, which I may call the carbamic acid theory, I shall confine myself to a short account of the experimental work that has established this theory in its present position. Twenty-five years ago Schultzen and Nencki* instituted a series of feeding experiments in order to learn whether the amido acids such as glycocoll, leucin and tyrosin, which are always to be found among the decomposition products of proteids and proteid-like bodies

* Berichte d. deutsch. chem. Gesell., 1869, p. 566–71, and Zeitschr. f. Biolog., Bd. 8, p. 124.

when they are subjected to hydrolytic decomposition,* had any connection with the formation of urea. They found that the nitrogen of these amido-acids, especially of glycocoll and leucin, reappeared in the urine in the form of urea, and this observation first suggested that carbamic acid is formed in the economy as an intermediate product in the course of their oxidation to urea. Salkowski next demonstrated† that when taurin is given to human beings it reappears to some extent in the urine as a salt of an acid called by him tauro-carbamic, but more properly named uramido-isæthionic acid, since its formula obliges us to look upon it as one of the uramido-acids, in other words, as a substituted urea.

Thus, $\begin{vmatrix} CH_2.NH(CONH_2) \\ CH_2.SO_3H \end{vmatrix}$ = amido-isæthionic acid. The formation of this compound in the body must, however, be looked upon as supporting the opinion that carbamic acid really exists in the economy, and that it can combine with certain compounds that have been introduced.

Schultzen's‡ experiments in 1872 on the fate of sarkosin in the organism of the dog, which were for a long time looked upon as having furnished strong probable evidence or even proof that certain amido-acids play an important rôle as precursors of urea, were afterward shown by Baumann, v. Mering,§ Salkowski‖ and Schiffer** to be far from conclusive for this theory.

A perusal of the papers of the investigators just named, especially of the historical introduction to Schiffer's paper, will give an idea of the interest aroused by Schultzen and Nencki's work, and of the laborious researches that have since been devoted to the fate in the body of various amido-com-

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* Tyrosin is not found among the decomposition products of the glutinoids, and glycocoll, which is regularly found among the decomposition products of gelatin, is not found among those of the proteids proper.

† Berichte d. deutsch. chem. Gesellsch., 1873, 6, pp. 744–6, pp. 1191–3 and 1312.

‡ Ber. d. deutsch. chem. Gesellsch. 1872 (5), pp. 578–81.

§ Berichte d. deutsch. chem. Gesellsch. 1875 (8), pp. 584–9.

‖ Zt. f. physiol. Chemie, Bd. IV., pp. 55–85, and pp. 100–33 ; also Ber. d. d. chem. Gesell. 1875, Bd. 8, pp. 638–40.

** *Ibid.* Bd. V., pp. 257–66, and Bd. VII., pp. 479–87.

pounds, researches which, it was hoped, would throw light on the formation of urea in the body.

The result of all this work on the amido-acids was that the carbamic acid origin of urea could not be accepted as demonstrated. But meanwhile (1875) appeared Drechsel's* notable paper already referred to, on the oxidation of glycocoll, leucin and tyrosin, and on the occurrence of carbamic acid in the blood. Drechsel demonstrated that when these compounds are oxidized in ammoniacal solution with ammonium permanganate, carbamic acid is always to be found among the oxidation products. Urea is not formed. Aqueous solutions of glycocoll oxidized with potassium permanganate also yield carbamic acid, and it is thus demonstrated that carbamic acid can be produced when there is no ammonia originally present. Otherwise it might have remained an open question whether the carbamic acid formed in the ammoniacal solutions of the first experiments was not the result of the interaction of nascent CO_2 with the ammonia originally present. In this paper Drechsel also furnished proof of the existence of a salt of carbamic acid in the blood of the dog. These results both chemical and physiological were challenged by Hofmeister,† who claimed to have demonstrated that the reactions obtained by Drechsel were to be referred to other substances than carbamic acid—the presence of unprecipitated calcium carbonate, oxaminic acid, etc. Drechsel‡ afterward repeated with great care those parts of the research upon which doubt had been thrown, and demonstrated to the satisfaction of workers in this field that his original position was well taken. Some years later Drechsel § again made an important experimental contribution to this subject by effecting the dehydration of ammonium carbamate to urea

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* *Loc. cit.* pp. 417–26.

† Journal f. prakt. Chemie (2), Bd. XIV., pp. 173-83, and Pflüger's Archiv, Bd. XII., p. 337.

‡ Journal f. prakt. Chemie (2), Bd. XVI., p. 169. See also D.'s important paper on the preparation and description of a number of new salts of carbamic acid, *ibid.* pp. 180–200, for valuable points bearing on the properties of carbamic acid, and notably of its calcium salt.

§ Journal f. prakt. Chemie (2), Bd. XXII., pp. 476–88; Archiv f. (Anat. u.) Physiol., 1880, p. 550.

in an aqueous solution by means of a rapidly interrupted electric current, thus imitating the conditions under which urea is formed in the human body more closely than when its precursors are heated to high temperature in sealed tubes, etc. The formation of urea by this electrolytic method is the result of an alternate oxidation and reduction. Now the living protoplasm of the body cells is endowed with oxidizing and reducing powers, and although the exact chemical explanation of these processes is not at hand, we have only to assume that these powers are in some instances exercised in an alternating manner to give Drechsel's experiments a wide application.

From our present knowledge of the locality in which urea is mainly formed, thanks to the brilliant experimental researches of W. von Schröder,[*] we may think of the liver cell as exercising an oxidizing and reducing action on the carbamate of ammonia, with the resulting formation of urea. These processes may be expressed in the following terms:

I. $NH_2.CO.O.NH_4 + O = NH_2.CO.O.NH_2 + H_2O.$
II. $NH_2.CO.O.NH_2 + H_2 = NH_2.CO.NH_2 + H_2O.$

With the help of this principle of an alternating oxidation and reduction, first definitely stated and copiously illustrated by Drechsel, many of the hitherto inexplicable chemical phenomena of the body become clear to us. Such are the many synthetic processes accompanied by water abstraction and the reductions that so frequently interrupt the step-by-step oxidations that are continually going on, and which lead to the formation of a large number of interesting intermediate products.[†]

Further experimental evidence of the existence of carbamic acid in the economy was furnished by Drechsel and Abel in their discovery of its occurrence as a normal product in the urine of the horse. The very interesting and novel experiments recently performed by Hahn, Massen, v. Nencki and

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[*] Archiv f. exp. Pathol. u. Pharmakol., Bd. XV., pp. 364–402, and *ibid.* Bd. XIX., pp. 373–86.

[†] Drechsel, Archiv f. (Anat. u.) Physiol. 1891, pp. 251–4. Nencki, Archiv f. exp. Pathol. u. Pharmakol., Bd. 32, p. 206. Baumann, Zeitschrift f. phys. Chem., Bd. XV., pp. 276–7.

Pawlow* have shown most convincingly that this acid has the importance that has for some time been attributed to it as an intermediate product in the breaking down of proteids within the body. These experimenters performed v. Eck's operation on dogs, that is, they tied the portal vein close to its entrance into the liver and established a free communication between this vessel and the inferior vena cava, so that all the blood from the portal district passed directly into the inferior vena cava and was entirely excluded from the liver. I must refer to the original treatise for the details of the operation, for the variations in the experiment, such as the partial or total resection of the liver, ligature of the hepatic artery in addition to the establishment of Eck's fistula, and for the details of the large amount of chemical work contained in this valuable contribution. After a variable period of time the animals thus operated upon manifested grave symptoms referable to the nervous system. There appeared a stage of somnolence with ataxia, followed by one of excitation with ataxy, analgesia and amaurosis, this again passing into a stage of clonic and tetanic convulsions, which were followed by coma sometimes terminating fatally. Chemical analyses demonstrated the presence of ammonium carbamate in largely increased quantity in both the blood and urine of those animals that had the nervous seizures just referred to. Pharmacological experiments also demonstrated a very satisfactory agreement in the symptoms of these nervous seizures resulting from the establishment of an Eck's fistula, with those observed in healthy dogs after the intravenous injection of very large doses of sodium carbamate (0.3–0.6 gram pro kilo). A differential test of great value is seen in the fact that a healthy animal is not poisoned by the administration of large doses of sodium carbamate by the mouth after previous neutralization of the hydrochloric acid of the stomach, whereas an animal that has been operated upon responds at once to this method of administration with the severe symptoms already referred to. It is evident, then, that in this latter case the greatly damaged liver is no longer able to convert with sufficient rapidity the carbamic acid absorbed from the digestive tract into the harm-

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* Archives des Sciences Biologiques, t. I., no. 4, pp. 401–97, and Archiv f. exp. Path. und Pharmak., Bd. 32, pp. 161–211.

less urea. Meat-eating has for dogs with an Eck's fistula the same consequences as the administration of a carbamate by the mouth. Interpreting their experiments in the light of previous knowledge as to the occurrence of carbamic acid and as to the functions of the liver in the synthesis of urea, Hahn, Massen, v. Nencki and Pawlow conclude that ammonium carbamate is formed everywhere in the tissues as the final stage of the decomposition of proteids and that it is carried to the liver and there converted into urea.

It would take us beyond the scope of this paper to discuss the chemical processes antecedent to the appearance of carbamic acid in the tissues, or how much urea is formed in other ways than by passing through the carbamic acid stage, or to raise the question as to the bearings upon the carbamic acid theory of the increased excretion of ammonia that has been observed in the terminal stages of hepatic cirrhosis, diabetes mellitus, etc. When it is borne in mind that carbamic acid makes its appearance whenever nitrogenous principles such as occur in the body are oxidized in alkaline media, that its salts occur in the blood and urine of animals and in the urine of human beings, that it can be converted into urea in the laboratory by simple chemical processes not foreign to the body, that it will yield urea if it be conducted through a "surviving" liver, and that it appears in increased quantity in the blood and urine under experimental conditions in which the functions of the liver have been deranged, it will be admitted that we have good reasons for believing that it bears an important relation to urea, and that its study in normal and pathological conditions must be of interest and value.